Rhinovirus infections: induction and modulation of airways inflammation in asthma

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Summary

There is renewed interest in the role of respiratory virus infections in the pathogenesis of asthma and in the development of exacerbations in pre-existing disease. This is due to the availability of new molecular and experimental tools. Circumstantial evidence points towards a potentially causative role as well as to possibly protective effects of certain respiratory viruses in the cause of allergic asthma during early childhood. In addition, it now has become clear that exacerbations of asthma, in children as well as adults, are mostly associated with respiratory virus infections, with a predominant role of the common cold virus: rhinovirus. Careful human *in vitro* and *in vivo* experiments have shown that rhinovirus can potentially stimulate bronchial epithelial cells to produce pro-inflammatory chemokines and cytokines, may activate cholinergic- or noncholinergic nerves, increase epithelial-derived nitric oxide synthesis, upregulate local ICAM-1 expression, and can lead to nonspecific T-cell responses and/or virus-specific T-cell proliferation. Experimental rhinovirus infections in patients with asthma demonstrate features of exacerbation, such as lower airway symptoms, variable airways obstruction, and bronchial hyperresponsiveness, the latter being associated with eosinophil counts and eosinophilic cationic protein levels in induced sputum. This suggests that multiple cellular pathways can be involved in rhinovirus-induced asthma exacerbations. It is still unknown whether these mechanisms are a distinguishing characteristic of asthma. Because of the limited effects of inhaled steroids during asthma exacerbations, new therapeutic interventions need to be developed based on the increasing pathophysiological knowledge about the role of viruses in asthma.

Keywords: airway hyperresponsiveness, asthma exacerbation, common cold, immune responses, respiratory virus infection

Introduction

Considering the rapid progress in the present research on the pathogenesis, pathophysiology, and immunopathology of asthma and chronic obstructive pulmonary disease (COPD), it is remarkable that the understanding of the potential involvement of respiratory virus infections in these diseases is far from settled. This seems to be due to the only recent availability of data from (i) epidemiological studies using modern virological techniques, and (ii) experimental studies in animals as well as in man. These approaches are increasing our knowledge on the potential role of respiratory

viruses in the onset of asthma and COPD as well as in inducing exacerbations in pre-existing disease.

Respiratory viruses in asthma and COPD

In general, the information on the complex involvement of respiratory virus infections in the pathogenesis of asthma is increasing. It shows circumstantial evidence of a broad spectrum of effects: between a potentially causative or facilitatory role [1,2] and an inhibitory or protective influence [3,4], probably depending on the type and age of infection. With regard to the importance of respiratory virus infections in exacerbations of pre-existing asthma, research during the past decade has provided clear positive evidence, from both epidemiological and experimental

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studies [5,6]. This is less clear in COPD. Latent virus infections have been implicated in its pathogenesis [7], but this still needs confirmation. Furthermore, there are only very recent data on a potentially (limited?) role of respiratory viruses in causing exacerbations of pre-existing COPD [8].

It is likely that respiratory viruses can have pro-inflammatory and immunomodulatory effects within the airways [9]. This has most extensively been studied for respiratory syncytial virus (RSV) [10,11] and for human rhinovirus [5,6,12]. This review will focus on the latter, and will particularly discuss the association between the pro-inflammatory and pathophysiological effects of rhinoviruses in patients with asthma.

Rhinovirus and asthma exacerbations

There is little doubt that rhinoviruses are important as a trigger of asthma exacerbations. Several clinical and epidemiological studies have described a close temporal association of respiratory virus infections with exacerbations in patients with asthma [13]. Respiratory viruses can be identified in 10%–44% of the asthma exacerbations in adults [14,15], whilst in children identification rates vary from 26% to 83% [16–19]. The use of the sensitive polymerase chain reaction technique to detect rhinovirus and coronavirus in the two most recent studies has resulted in the highest identification rates so far [15,19]. Among the various respiratory viruses identified, rhinovirus predominates in most of these studies, accounting for about 50% of the detected viruses [15–19]. The incidence of rhinovirus infections may even be higher in asthmatic patients as compared with nonasthmatic subjects [13,20], although rhinovirus shedding in the absence of cold symptoms does not seem to be associated with clinical worsening of asthma [17]. Taken together, these findings indicate that rhinovirus infections may have a causal role in exacerbations of asthma. Preliminary data confirm that this also occurs in exacerbations of COPD, but to a lesser degree [8].

Experimental rhinovirus infections in asthma

The observation that common colds are important in inducing exacerbations in asthma, offers the perspective of experimental studies that can be safely performed in humans *in vivo* [21]. Indeed, experimental infections have been shown to be a useful tool for investigating the effects of rhinovirus infections in allergic disease or asthma [22–35]. Such a model allows careful patient selection and monitoring, and intensive assessment of the rhinovirus-induced effects, under controlled circumstances and timing. Thus, the effects of a rhinovirus infection can be assessed at the level of asthma symptoms, changes in use of

asthma medication, lung function, and airway hyperresponsiveness, as well as the underlying airway pathology.

Physiological effects

So far, experimental rhinovirus infections in patients with asthma and/or atopic rhinitis have not been shown to induce a significant change in lung function, e.g. FEV₁, when measured during laboratory visits [22,24–26,28,29,31,35]. This has been considered to be reassuring in terms of patient-safety of the experimental model; however, recent results from our laboratory are demonstrating that frequent home-recordings of FEV₁ (three times daily) do decrease in atopic asthmatic patients in the acute phase of an experimental RV16 infection [27]. Furthermore, the maximal decrease in FEV₁ in the acute phase of the infection, expressed as percentage of the recent personal best, correlated significantly with the observed increase in airways hyperresponsiveness [27]. This suggests that there is transient worsening of airways obstruction after rhinovirus infection in asthma, which may improve spontaneously during the day, or as a consequence of repeated deep-breath manoeuvres as are being performed in the lung function laboratory. This points at either increased sensitivity to bronchoconstrictive stimuli and/or a reduced bronchodilating effect of a deep breath [36].

The effects of experimental rhinovirus infection on airway responsiveness vary among different studies, using different rhinovirus serotypes. Lemanske *et al.* and Gern *et al.* have demonstrated an enhanced hypersensitivity to histamine and allergen challenge after experimental rhinovirus 16 (RV16) infection in nonasthmatic patients with atopic rhinitis [24,34], which was significantly different from the lack of response in normals [34]. Others, however, have not observed such an effect when using rhinovirus 39 [35]. In asthmatic subjects, Halperin *et al.* has found increased hypersensitivity to histamine in only four of 22 subjects after experimental rhinovirus (serotype 39 and HH strain) infection [22]. More recently, in our laboratory Cheung *et al.* have shown that RV16 increases asthma symptoms, coinciding an increase in the maximal bronchoconstrictive response to methacholine up to 15 days after infection [25], pointing towards the development of excessive airway narrowing due to a rhinovirus infection. In a similar design, we have shown a significant increase in airway sensitivity to histamine in asthmatic subjects after RV16 infection, which was most pronounced in those subjects who had severe cold symptoms [28]. Taken together, these data indicate that patients with asthma and/or atopic rhinitis may suffer more prominent pathophysiological consequences from a rhinovirus infection as compared with nonatopic nonasthmatic subjects, suggesting an interactive effect of virus-induced airways inflammation with

features of the underlying disease, such as altered airway geometry [37,38] and/or pre-existing allergic sensitization/inflammation [39].

Animal studies

Experimental animal studies have provided strong evidence of the induction of airways inflammation by respiratory viruses, as is apparent from epithelial shedding and morphological changes after, for example, parainfluenza infection in guinea pigs [40]. It has not, however, been resolved to what extent such induced inflammation contributes to the accompanying physiological changes. Initial findings suggested a major role of inflammatory cells, such as T cells, basophils, and their mediators [41]. And indeed, virus-induced airway hyperresponsiveness could be transferred by bronchoalveolar lavage cells recovered after viral infection in guinea pigs [42]. However, more recent results obtained by using antibodies against pro-inflammatory cytokines, such as IL-5, are indicative of (partly) independent induction of airways hyperresponsiveness and eosinophilic airways inflammation by respiratory viruses in guinea pigs *in vivo* [43].

Apparently, neurogenic mechanisms can also be responsible for virus-induced hyperresponsiveness in animals. Interestingly, this seems to be mainly due to impaired inhibitory mechanisms [44]. Such impaired neurogenic protection might arise from reduced production or activity of the neuropeptide degrading enzyme neutral endopeptidase [45], or to dysfunction of inhibitory cholinergic M₂-receptors [46]. In addition, there is evidence that virus-induced airways hyperresponsiveness in guinea pigs can be caused by impaired synthesis of nitric oxide (NO) [47]. This is potentially important, since it has recently been shown that rhinovirus can induce NO-synthase expression in human primary bronchial epithelial cells *in vitro* [48], and that such NO can reduce rhinovirus replication and the induced inflammatory cytokine release by epithelial cells [49]. Interestingly, our latest data in asthmatics *in vivo* are in keeping with such a protective mechanism by NO after rhinovirus infection (Fig. 1) [50], but this hypothesis remains to be tested using appropriate pharmacological blocking agents in asthmatics *in vivo*.

Inflammatory response in humans

What do we know about the inflammatory response to a rhinovirus infection in asthmatic and/or atopic rhinitis patients? It is likely that epithelial cells are playing an active and major role in this. *In vitro*, there is evidence that infection with rhinovirus in pulmonary epithelial cells and fibroblasts induces *de novo* synthesis of pro-inflammatory cytokines, such as GM-CSF, IL-11, and IL-6, and the

Fig. 1. Relationship between the change in exhaled NO and the change in airway hyperresponsiveness to histamine (PC₂₀) during the first week after experimental infection with RV16 in patients with asthma *in vivo*. The patients with the greatest increase in airway hyperresponsiveness (reduction in PC₂₀) are showing the lowest increase in exhaled NO and *vice versa*. The regression line has been drawn. Rs, Spearman rank correlation coefficient. Duplicated with permission from [50].

chemokines IL-8 and RANTES [51–55] by activating transcription factors such as NF- κ B [53,56,57]. Such epithelial chemokine release appears to be prolonged until 5 days after *in vitro* infection [58]. Indeed, during common colds *in vivo*, these mediators and others were found to be elevated in nasal secretions [28,57,59–67] as well as in sputum (IL-8, IL-6) [30,68].

In view of the chemotactic properties of the above-mentioned chemokines on lymphocytes, basophils, neutrophils, and (primed) eosinophils, one can postulate that such chemokines drive the secondary recruitment of inflammatory cells [69]. Indeed, a rhinovirus infection is known to lead to infiltration of leucocytes, particularly neutrophils and mononuclear cells, into nasal secretions [68,70–72] rather than the nasal mucosa [59,70,73]. Both in normals and asthmatic subjects this occurs in conjunction with an increase in the number of neutrophils in peripheral blood, whereas the number of circulating lymphocytes falls in the acute phase of infection [25,26,28,74]. These effects, as well as the decrease in PC₂₀ in asthmatic subjects, are significantly related to the severity of the cold, as reflected by the cold score [28,74] and the increase in IL-8 in nasal lavage [28], underlining the relationship between the severity of the cold and the pathophysiological consequences [17,28].

In contrast to the findings in the nose, the only available study so far on the effects of a common cold on intrapulmonary airways inflammation showed that in the bronchial biopsies the numbers of T lymphocytes in the submucosa and eosinophils in the epithelium were elevated both in normal and/or in atopic asthmatic subjects following RV16 infection [26]. Interestingly, the numbers of eosinophils remained elevated up to 6 weeks after infection in the six asthmatic subjects [26]. This is in keeping with the increased levels of eosinophilic cationic protein (ECP) in sputum that we have observed after experimental RV16 infection in asthmatics, which correlated significantly with the increase in airway sensitivity to histamine (Fig. 2) [30]. One could speculate that local production of eosinophil chemotactic chemokines contributes to virus-induced eosinophilic airways inflammation in asthma.

Involvement of intercellular adhesion molecule-1 (ICAM-1)

ICAM-1 has been the focus of much attention in rhinovirus-induced inflammation because of its dual role as (i) an adhesion molecule on endothelial cells or epithelial cells for binding of infiltrating leucocytes such as eosinophils and lymphocyte subsets [75–78], and (ii) as the major rhinovirus receptor [79,80]. It appears that ICAM-1 expression is up-regulated upon stimulation with rhinovirus in pulmonary epithelial cell lines *in vitro* [55,56]. This has now been confirmed *in vivo* in a recent study from our laboratory demonstrating that experimental rhinovirus colds in asthmatics increase epithelial ICAM-1 expression in bronchial biopsy specimens [81]. Hence, up-regulation of ICAM-1 by secondarily released cytokines such as IL-1 β and TNF- α , may facilitate rhinovirus infection in susceptible cell types [52,55,82].

ICAM-1-binding to its ligands [77], lymphocyte function associated antigen (LFA-1, CD11a/CD18), and macrophage-1 antigen (MAC-1, CD11b/CD18), is involved in the process of leucocyte adherence and migration, and in addition, may provide costimulatory signals for CD4 cell and lymphokine-activated killer cell activation, T-cell mediated cytotoxicity, and T-cell dependent B-cell activation. It has been postulated that cytokines such as TNF α , IL-1 β , IFN- γ , and IL-4 may alter the expression, function, or configuration of ICAM-1, thereby specifically promoting (TNF α , IFN- γ , IL-4) or impeding (IL-6, IL-1) lymphocyte adhesion to, for example, cytokine-pretreated fibroblasts or primary tracheal epithelial cells [52,83,84]. Rhinovirus binding, however, does not seem to be affected by such cytokine-induced functional changes [52,83,84], suggesting that virus-induced pro-inflammatory cytokines may facilitate infection, whilst promoting or inhibiting the interaction between epithelial cells and lymphocytes.

Fig. 2. Relationships between the change in percentage of eosinophils in induced sputum (a) or in levels of ECP in supernatant of induced sputum (b) and the change in airway hyperresponsiveness to histamine (PC₂₀) in the first week after experimental RV16 infection in patients with asthma *in vivo*. Even though there were no significant virus-induced changes in percentage eosinophils or ECP levels, the observed changes in these variables were significantly related to those in PC₂₀: the greater the development of airways hyperresponsiveness (decrease in PC₂₀), the greater the increase in percentage of sputum eosinophils or ECP levels. The regression lines have been drawn; r , correlation coefficient. Duplicated with permission from [30].

Immune response

What are the immunological changes following rhinovirus infection? Incubation of either infectious or inactivated rhinovirus particles with a mixture of antigen presenting cells (APC; such as monocytes and also eosinophils [78]) and lymphocytes can induce a proliferative response to rhinovirus [85], while concomitantly hampering the monocytes-induced, ICAM-1-mediated, specific T-cell proliferation and cytotoxicity to other

antigens [86]. This indicates that binding of rhinovirus to ICAM-1 may interfere with ICAM-1/LFA-1 interaction [86]. Indeed, the ICAM-1 binding sites for LFA-1 and rhinovirus overlap partially [87]. Natural killer cell activation and cytotoxicity, which is less dependent on ICAM-1/LFA-1 interaction, was not shown to be affected, or even increased after *in vitro* rhinovirus inoculation [86,88,89].

Rhinovirus does not replicate inside monocytes and airway macrophages [90]; however, the uptake of infectious rhinovirus particles (either mediated or not mediated by ICAM-1) by APCs may increase nonspecific T cells activation [91]. This is reflected by the increased expression of the activation marker CD69, but not CD25 on the cell membrane [91], and spontaneous [85] or mitogen-induced secretion of IL-2 and IFN- γ in normal subjects [88,91]. In addition, from 3 weeks after infection onward, antigen-specific lymphocyte proliferation could be demonstrated [88,92,93], indicating a cell-mediated immune response to the infection [85]. Such CD4⁺ cells may be specific to either serotype-restricted or serotype-shared viral epitopes [85,94,95]. A recent report suggests that eosinophils, when expressing ICAM-1, may function as APCs, in that they induce T-cell proliferation when incubated with rhinovirus [78]. This is associated with an increase in CD18 expression (an ICAM-1 ligand), thus providing evidence for a possible positive feedback loop in rhinovirus-induced eosinophil activation within the airways.

In a comparative study, using *in vivo* experimental RV39 infection, patients with allergic rhinitis appeared to have lower numbers of T-helper cells (either activated or not activated) and RV39-induced peripheral blood mononuclear cell proliferation as compared with normals [93], while only in the rhinitis patients was an increase in proliferation to RV39 noted as early as during the acute phase of the infection. Furthermore, the decrease in NK-activity was less marked in the rhinitis patients as compared with the normals [93]. This suggests that there may be disease-related differences in cell-mediated immunity, although it is still unclear as to how such differences might be interpreted in terms of mechanisms of rhinovirus-induced exacerbations of asthma.

Infection of the lower airways?

Is rhinovirus actually present within the lower airways? In the nasopharynx, rhinovirus replicates in the adenoid tissue and the epithelium [96], and can readily be detected in tissue culture or by reverse transcriptase-polymerase chain reaction [97]. Evidence of rhinovirus infection of the intrapulmonary airways is more difficult to obtain. Although rhinoviruses can occasionally be cultured from sputum [98], tracheal brushings [31], and bronchoalveolar lavage (BAL) fluid [33,99], possible nasopharyngeal contamination

precludes definite conclusions as to virus infection of the lower airways. The use of reverse transcriptase-polymerase chain reaction on BAL cells, rather than BAL fluid, has increased the likelihood of detecting lower airways infection, and has led to a detection rate of 80%, while the detection rate in nasal lavage fluid in the same subjects was 100% after experimental RV16 infection [100].

In view of the difficulties in detecting rhinoviruses in the lungs so far, and the relative fastidiousness of rhinoviruses for culture condition (particularly the relatively low optimal culture temperature of 33 °C), a high grade infection of the lower airways may not be very likely. One could speculate, however, that host factors such as increased ICAM-1 expression in the nasal epithelium [101] might increase the susceptibility of asthmatic and/or atopic patients to symptomatic rhinovirus colds [20], even after repeated infection with the same serotype [23,28,102]. Similarly, increased expression of ICAM-1 in the lungs [56,75] could promote lower airways infection, and its pathophysiological consequences. Apparently, *in situ* techniques to detect the viral genome [103–105] will need to be applied in order to conclusively assess viral contamination and/or infection within the lower airways. Preliminary results using *in situ* hybridization on bronchial biopsy specimens obtained from normals and asthmatics after experimental infection may indeed be suggestive of the possibility that rhinovirus can infect the lower airways [106]; however, confirmation of these results has to be awaited.

Conclusions

There is little doubt that respiratory virus infections can have pro-inflammatory effects within the airways. Whilst it is still unresolved as to whether this contributes to the onset of asthma, there is convincing evidence that rhinovirus infections in particular are associated with exacerbations of the disease. Rhinovirus infection is able to activate bronchial epithelial cells, leading to the release of pro-inflammatory cytokines (chemokines) as well as of modulating substances (such as NO). This can be accompanied by virus-specific T-cell proliferation weeks after infection, at early stages preceded by nonspecific T-cell responses that could contribute to virus-induced airways inflammation. By up-regulating ICAM-1 expression on, for example, epithelial cells and eosinophils, there is the possibility of positive feedback loops between rhinovirus infection and the inflammatory response.

Some of these events can be observed following experimental rhinovirus infection in patients *in vivo*. These are accompanied by clinical symptoms, airways obstruction, and worsened airway hyperresponsiveness. The latter is associated with changes in eosinophil counts and levels of ECP in induced sputum. This suggests, but does not prove,

that eosinophilic inflammation can be involved in rhinovirus-induced airways inflammation and the associated exacerbations of asthma.

In general, the clinical, physiological, and cellular responses to experimental rhinovirus infections in patients with asthma are relatively mild. This underlines the safety of this procedure, but may not adequately mimic the events occurring after a natural common cold. It can be postulated that this requires more complex experimental models, such as those with a pre-existing flare-up of allergic inflammation with, for example, up-regulated ICAM-1 [107]. This might be obtained by a design in which rhinovirus inoculation follows repeated low-dose allergen exposure [108,109]. This may mimic the course of events that occur during spontaneous exacerbations more accurately [110]. It is hoped that such experiments will bring us a therapy for virus-induced acute exacerbations of asthma that is more successful than the ones currently available [111,112].

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